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Previews

RNA in the Loop

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Long noncoding RNAs (lncRNAs) have been implicated in a variety of biological roles, particularly as *cis* or *trans* gene expression regulators. Reporting recently in *Nature*, Lai et al. (2013) show that a class of gene-activating lncRNAs combines two gene regulation paradigms: enhancer-directed chromosomal looping and RNA-mediated protein effector recruitment.

For decades, we have known that haploid genome size (the C-value) does not correlate well with perceived organismal complexity. The discovery that much of the genome consists of non-protein-coding "junk DNA" (so named for the overwhelming burden of genomic parasites and evolutionary relics) partially resolved this paradox, but there remains a "C-value enigma" as to what accounts for the wide variations in noncoding content and what, if any, functions they perform. It was realized early on that much of this noncoding space produces RNA, and recent advances in high-throughput technologies suggest as much as 75% of the genome might be "pervasively transcribed" (Djebali et al., 2012). However, it remains controversial whether most of these noncoding RNAs serve biological purposes or are merely products of an inherently noisy genome (Kung et al., 2013).

Hints of possible function for lncRNAs (noncoding transcripts >200 nt) emerged in the early 1990s with the discoveries of *H19* and *Xist*, implicated in regulation of genomic imprinting and X chromosome inactivation (XCI), respectively. Both of these processes lead to allele-specific gene expression. In the following decade, many more lncRNAs have been identified within the X-inactivation center and imprinted gene clusters, leading to the suspicion that lncRNAs may be a general property of *cis*-regulatory phenomena. lncRNAs have since been found to be involved in a variety of developmental or disease processes, from regulation of pluripotency to cancer. However, much work is still needed to elucidate the mechanisms of action for most of these lncRNAs, answering questions such as how the RNA is targeted to its specific locus of action, whether function depends on the transcript or the act of transcription, and whether the RNA functions as an lncRNA per se or as a source of small ncRNAs or short peptides.

One common theme seen repeatedly among well-studied lncRNAs is that they act as scaffolds for targeting gene regulatory proteins. RNAs such as *Xist*/*RepA*, *Tsix*, *HOTAIR*, and *ANRIL* have been found to bind one or more repressive epigenetic factors such as polycomb repressive complexes (PRC1 and PRC2), LSD1, and DNA methyltransferases (Kung et al., 2013; Wang and Chang, 2011). While many of these examples involve lncRNAs as repressors, some lncRNAs instead activate gene expression. For instance, *Jpx* has been identified as a *trans* activator of *Xist* expression (Tian et al., 2010). In a recent publication in *Nature*, Lai et al. (2013) report that a set of lncRNAs called ncRNA-activators (ncRNA-a) may exert their gene-activating function by interacting with the Mediator, a key transcriptional coactivator complex.

lncRNAs could either act *in trans*, regulating distant genes from the lncRNA's locus, or *in cis*, regulating genes proximal to the lncRNA's site of synthesis. *Trans*-targeting mechanisms remain mostly unknown. On the other hand, *cis*-acting RNAs such as *Xist*/*RepA* are thought to cotranscriptionally recruit and tether protein effectors. lncRNAs are uniquely suited to act as allele- and locus-specific recruiters (Figure 1A) by virtue of their length (allowing them to reach out and capture protein factors while tethered to chromatin), their specificity (since most lncRNAs emanate from single loci), and the fact that they are inherently hybridized to chromatin through DNA:RNA heteroduplexes during transcription (Kung et al., 2013).

An additional facet of lncRNA functional mechanism is revealed by studies employing chromosome conformation capture (3C). The *cis*-acting *HOTTIP* RNA has been found to recruit the gene-activating Trithorax group complex MLL to coordinately regulate loci in the *HOXA* cluster, as far apart as 40 kb, that have been brought into close proximity in 3D through long-range chromosomal interactions (Wang and Chang, 2011). Mammalian enhancer elements have long been hypothesized to play a role in such long-range interactions, sometimes exerting their activating effects hundreds of kilobases from their gene targets (Krivega and Dean, 2012). In 2010, Mediator and cohesin together were found to be responsible for the formation of cell-type-specific long-range interactions between enhancers and the core promoters of target genes (Kagey et al., 2010). Mediator thus acts as a bridge between transcription factors binding at distant enhancers and the RNA polymerase II (RNAPII) apparatus at target promoters.

The association of lncRNA with mammalian enhancers has been noted for some time, further highlighted by recent studies demonstrating functional noncoding transcription from certain neuronal enhancers (Bond et al., 2009; Onodera et al., 2012) and a class of activating lncRNAs called "ncRNA-a" (Ørom et al., 2010). In particular, ncRNA-a are associated with expression of protein-coding genes within 300 kb of their loci, such that their depletion leads to downregulation of target genes. Reporter assays

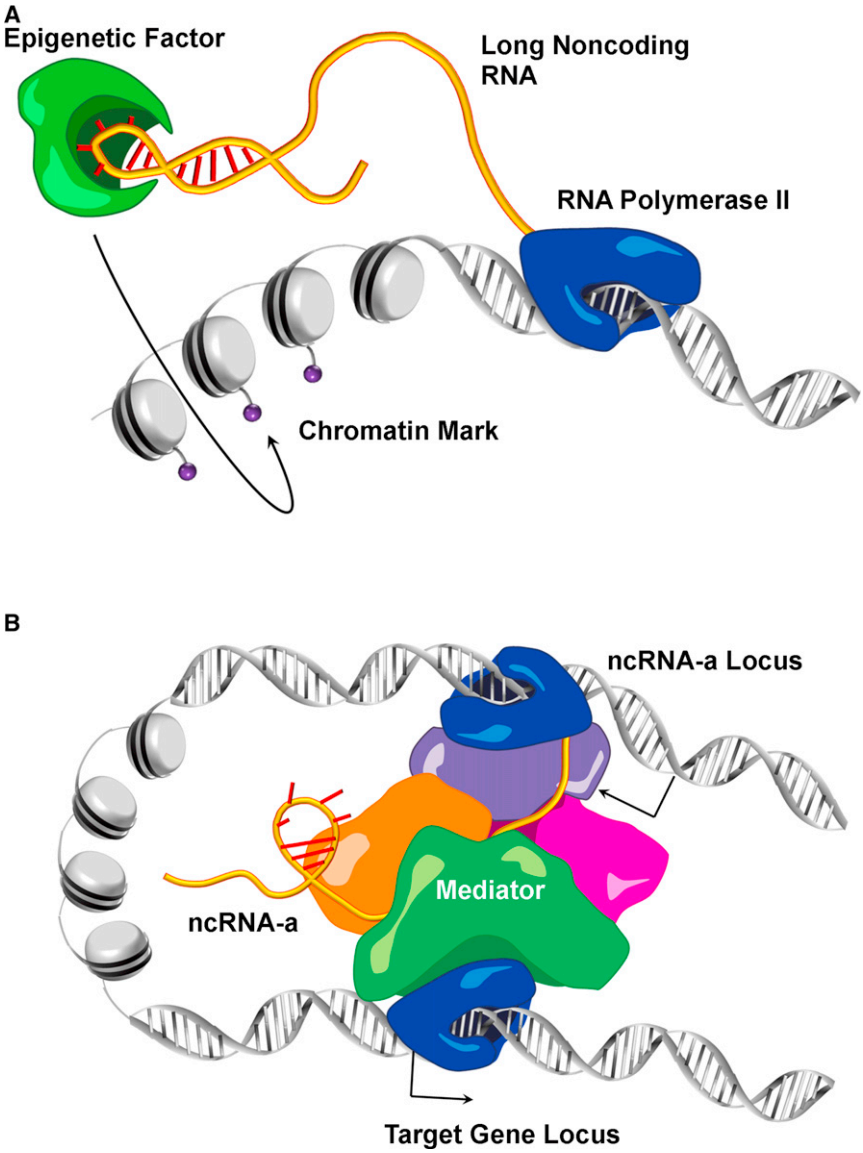

Figure 1. Schematics of lncRNA-Directed *cis*-Regulatory Mechanisms
(A) Tether model of *cis* regulation by lncRNA. An lncRNA tethered to chromatin (in this case by RNAPII in the process of transcription) recruits an epigenetic factor to deposit chromatin marks at sites proximal to the noncoding locus.
(B) Model of ncRNA-a function as described by Lai et al. (2013) An ncRNA-a interacts with the multisubunit Mediator complex to facilitate the formation of a long-range DNA loop, bringing the enhancer-like ncRNA-a locus into physical proximity with its target locus. This then leads to robust expression of the target gene. Adapted from Kung et al. (2013). Credits to D. Colognori.

were used to find that the effects of ncRNA-a were independent of the RNA loci's orientation and require the target genes' own promoter, just like enhancers, while assays using truncated ncRNA-a transcription units or ones where the ncRNA promoter is attached to a protein-coding gene body confirm that the activating effect depends on the transcript, not just the act of transcription.

In the follow-up study by the Shiekhattar group, Lai et al. (2013) now propose a tantalizing link between enhancers that produce lncRNAs on one hand and Mediator's role as a bridge between enhancers and target genes on the other. Focusing on two of these activating lncRNAs, ncRNA-a3 and ncRNA-a7, they found that knocking down Mediator subunits (but not cohesin) compromises the activity of ncRNA-a in both reporter assays and at their native loci. At the same time, depletion of the ncRNAs leads to decreased localization of both Mediator and RNAPII to the target gene promoters. Using a variety of biochemical assays (including in vitro pulldown assays, UV-crosslinked RNA immunoprecipitation, and chromatographic fractionation), the group showed that ncRNA-a interacts directly with Mediator components. Of possible clinical interest is that two known mutations of Mediator subunit MED12, associated with the X-linked Opitz-Kaveggia syndrome, abolish Mediator-RNA interactions. In support of the DNA-looping model of enhancer action, 3C analyses reveal that ncRNA-a loci and their target genes are in physical proximity, and knocking down either Mediator or the ncRNA-a abrogates this interaction. Additionally, in vitro kinase assays and chromatin immunoprecipitation experiments suggest that ncRNA-a is required for Mediator's kinase activity specifically in phosphorylating histone 3 serine 10, an activating chromatin mark.

Taken together, the studies suggest that a class of mammalian lncRNAs function like enhancers to activate their target genes by interacting with the Mediator complex to establish long-range DNA looping (Figure 1B). While the common role of Mediator in facilitating such loop formation is characterized in both the Lai et al. (2013) and the Kagey et al. (2010) studies, the fact that cohesin knockdown did not affect ncRNA-a activity indicates that a different mechanism may be involved here, although it remains to be exhaustively tested if there is overlap between the sets of enhancers investigated in the two studies. In addition, the fact that knockdown of Mediator downregulated the expression of both ncRNA-a3 and its target, but not so in the case of ncRNA-a7, argues that ncRNA-a may not be a uniform class (especially since ncRNA-a3 forms a bidirectional pair with ncRNA-a4; Ørom et al., 2010). Finally, with increasing evidence that mammalian enhancers and promoters produce lncRNAs (Krivega and Dean, 2012), it may not be far-fetched to hypothesize that such RNA-mediated DNA looping is commonly employed for enhancer action. It could further be instructive to explore if other *cis*-regulatory genetic

elements, such as silencers and insulators, also function through a similar mechanism.

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Holding On and Letting Go: Cadherin Turnover in Cell Intercalation

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In zebrafish early development, blastoderm cells undergo extensive radial intercalations, triggering the spreading of the blastoderm over the yolk cell and thereby initiating embryonic body axis formation. Now reporting in *Developmental Cell*, Song et al. (2013) demonstrate a critical function for EGF-dependent E-cadherin endocytosis in promoting blastoderm cell intercalations.

Morphogenesis in developing animals is mediated by extensive rearrangements of cells. Typically, these rearrangements are mediated by combinations of cell migration and cell intercalation. In zebrafish embryogenesis, the first major morphogenetic processes are the bulging of the yolk cell toward the animal pole of the embryo (dome formation) and the spreading of the blastoderm over the yolk cell in the direction of the vegetal side (epiboly) (Figure 1). Both of these processes are thought to be driven by radial intercalation of blastoderm cells. Previous studies have suggested that radial intercalation of blastoderm cells is triggered by the graded distribution of E-cadherin from the outside to the inside of the blastoderm, promoting the movement of deep cells toward the blastoderm surface (Kane et al., 2005). However, while there is compelling evidence for E-cadherin being required for radial intercalation and epiboly movements of blastoderm cells, the existence and functional requirement of an E-cadherin gradient within the blastoderm remain controversial (Montero et al., 2005).

Song et al. (2013), in this issue of *Developmental Cell*, investigate maternal zygotic *spiel-ohne-grenzen* (MZ*spg*) mutant embryos, which carry a loss-of-function mutation in the Pou5f1/Oct4 gene and show defects in epiboly progression (Lachnit et al., 2008). By analyzing E-cadherin localization during epiboly, the authors find that in the blastoderm cells of epibolizing wild-type embryos, E-cadherin localizes to intracellular vesicles, which are positive for Rab-4, Rab-5, and Rab-11. In contrast, no such endosomal E-cadherin localization was visible in MZ*spg* mutant embryos. These observations suggest that internalization and/or recycling of E-cadherin are defective in MZ*spg* mutants and that the epiboly movement phenotype in mutant embryos might be due to this reduction in E-cadherin turnover. The authors also provide evidence that Pou5f1/Oct4 function in E-cadherin endocytosis is mediated by epidermal growth factor (EGF)-signaling triggering E-cadherin endocytosis and/or recycling. In MZ*spg* embryos, the expression of *EGF* mRNA is severely downregulated, and the overexpression of EGF restores subcellular E-cadherin vesicle formation and partially rescues the MZ*spg* epiboly delay phenotype. Furthermore, pharmacological inhibition of EGF receptor (EGFR) kinase activity decreases the number of E-cadherin vesicles and causes epiboly delay in wild-type embryos. Finally, the authors show that p120-catenin, previously shown to bind to E-cadherin and modulate E-cadherin turnover, exhibits more abundant plasma membrane localization in EGFR-inhibited embryos, suggesting that p120-catenin might mediate EGF-dependent E-cadherin internalization.